

**WHAT IS CLAIMED IS**

1. A method of determining the apparent affinity ( $K_d$ ) of binding between a PDZ domain and a ligand, comprising
- 5 (a) immobilizing a polypeptide comprising the PDZ domain and a non-PDZ domain on a surface;
- (b) contacting the immobilized polypeptide with a plurality of different concentrations of the ligand;
- (c) determining the amount of binding of the ligand to the immobilized polypeptide at each of the concentrations of ligand;
- 10 (d) calculating the apparent affinity of the binding from the binding determined in (c).
2. The method of claim 1, wherein the polypeptide is immobilized by binding the polypeptide to an immobilized immunoglobulin that binds the non-PDZ domain.
- 15 3. The method of claim 1, wherein the polypeptide comprising the PDZ domain is a fusion protein.
4. The method of claim 3, wherein the fusion protein is a GST-PDZ domain fusion protein.
- 20 5. A method of determining the  $K_i$  of an inhibitor or suspected inhibitor of binding between a PDZ domain and a ligand, comprising
- (a) immobilizing a polypeptide comprising the PDZ domain and a non-PDZ domain on a surface;
- (b) contacting the immobilized polypeptide with a plurality of different mixtures of the ligand and inhibitor, wherein the different mixtures comprise a fixed amount of ligand, at least a portion of which is detectably labeled, and different concentrations of the inhibitor;
- (c) determining the amount of ligand bound at the different concentrations of inhibitor;
- 30 (d) calculating the  $K_i$  of the inhibitor from the binding determined in (c).

6. The method of claim 5 wherein the polypeptide is immobilized by binding the polypeptide to an immobilized immunoglobulin that binds the non-PDZ domain.

7. The method of claim 6 wherein the fixed amount of ligand is between about 0.01 Kd and about 2 Kd.

8. A method of identifying an agent that enhances the binding of a PDZ domain to a ligand, comprising

(a) immobilizing a polypeptide comprising the PDZ domain and a non-PDZ domain on a surface;

(b) contacting the immobilized polypeptide with the ligand in the presence of a test agent and determining the amount of ligand bound; and,

(c) comparing the amount of ligand bound in the presence of the test agent with the amount of ligand bound by the polypeptide in the absence of the test agent,

wherein at least two-fold greater binding in the presence of the test agent compared to the absence of the test agent indicates that the test agent is an agent that enhances the binding of the PDZ domain to the ligand.

9. The method of claim 8 wherein the polypeptide is immobilized by binding the polypeptide to an immobilized immunoglobulin that binds the non-PDZ domain.

10. A method of determining the potency ( $K_{\text{enhancer}}$ ) of an enhancer of binding between a PDZ domain and a ligand, comprising

(a) immobilizing a polypeptide comprising the PDZ domain and a non-PDZ domain on a surface;

(b) contacting the immobilized polypeptide with a plurality of different mixtures of the ligand and enhancer, wherein the different mixtures comprise a fixed amount of ligand, at least a portion of which is detectably labeled, and different concentrations of the enhancer;

(c) determining the amount of ligand bound at the different concentrations of enhancer;

(d) calculating the potency ( $K_{\text{enhancer}}$ ) of the enhancer from the binding determined in (c).

11. The method of claim 10 wherein the polypeptide is immobilized by binding the polypeptide to an immobilized immunoglobulin that binds the non-PDZ domain.

12. The method of claim 11 wherein the fixed amount of ligand is between about  
5 0.01 Kd and about 0.5 Kd.

13. A method of identifying a high specificity interaction between a particular PDZ domain and a ligand known or suspected of binding at least one PDZ domain, comprising:

(a) providing a plurality of different immobilized polypeptides, each of said  
10 polypeptides comprising a PDZ domain and a non-PDZ domain;

(b) determining the affinity of the ligand for each of said polypeptides;

(c) comparing the affinity of binding of the ligand to each of said polypeptides,  
wherein an interaction between the ligand and a particular PDZ domain  
is deemed to have high specificity when the ligand binds an immobilized polypeptide  
15 comprising the particular PDZ domain with at least 2-fold higher affinity than to immobilized  
polypeptides not comprising the particular PDZ domain (a).

14. The method of claim 13 wherein the polypeptide is immobilized by binding the polypeptide to an immobilized immunoglobulin that binds the non-PDZ domain.

15. A method for determining the PDZ-PL inhibition profile of a compound  
comprising:

(a) providing

(i) a plurality of different immobilized polypeptides, each of said  
25 polypeptides comprising a PDZ domain and a non-PDZ domain;

(ii) a plurality of corresponding ligands, wherein each ligand  
binds at least one PDZ domain in (i);

(b) contacting each of said immobilized polypeptides in (i) with a  
corresponding ligand in (ii) in the presence and absence of a test compound;

(c) determining for each polypeptide-ligand pair in (b) whether the test  
30 compound inhibits binding between the immobilized polypeptide and the corresponding ligand  
thereby determining the PDZ-PL inhibition profile of the test compound.



27. A method for identifying a modulator of an interaction between a PDZ and a PL comprising carrying out the method of claims 23 in the presence and absence of a test compound and detecting a difference in at least one PDZ-PL interaction in the presence and absence of the test compound.

28. A method for identifying a modulator of an interaction between a PDZ and a PL comprising carrying out the method of claim 24 in the presence and absence of a test compound and detecting a difference in at least one PDZ-PL interaction in the presence and absence of the test compound.

29. The method of claim 27 wherein the modulator is an enhancer of the interaction.

30. The method of claim 27 wherein the modulator is an inhibitor of the interaction.